FIG. 1-1

**Constitutively Active Receptors**

| File Name | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CLASS A GROUP I . | | | | | |
| MSHR_mouse | melanocyte-stimulating hormone<br><br>MSH | TMII | 92<br>VSIVLETTIIL SEQ ID NO: 2<br>K | adenylyl cyclase activity/ HEK293, *stably transfected* | (Robbins, Nadeau et al. 1993) |
| CLASS A GROUP II<br>SH1B_human | 5-hydroxytryptamine$_{1B}$ | C-terminus of IC3 | 313<br>RERKATKTLGI SEQ ID NO: 3<br>K, R, Q | binding of [$^{35}$S]GTP[S] / CHO-KI | (Pauwels, Gouble et al. 1999) |
| 5H2A_human | 5-hydroxytryptamine$_{2A}$ | C-terminus of IC3 | 322<br>NEQKACKVLGI SEQ ID NO: 4<br>K | IP production / COS-7 | (Egan, Herrick-Davis et al. 1998) |
| 2H2C_rat | 5-hydroxytryptamine$_{2C}$ | C-terminus of IC3 | 312<br>NEDDASKVLGI SEQ ID NO: 5<br>L | PI hydrolysis / COS-7 | (Herrick-Davis, Egan et al. 1997) |

FIG. 1-2

| CLASS A GROUP II | | | | | |
|---|---|---|---|---|---|
| A1AB_human | $\alpha_{1B}$-adrenergic<br>alpha 1B-AR | TMDI<br>junction between TMDIII and IC2 | 63<br>FAIVGNILVIL SEQ ID NO: 6<br>A<br>142<br>CAISIDRYIGV SEQ ID NO: 7<br>A | IP / COS-7 | (Scheer, Fanelli et al. 1997) |
| A1AB_human | $\alpha_{1B}$-adrenergic<br>alpha 1B-AR | junction between TMDIII and IC2 | 143<br>CAISIDRYIGV SEQ ID NO: 8<br>K | IP / COS-7 | (Scheer, Costa et al. 2000) |
| A1AB_human | $\alpha_{1B}$-adrenergic | TMIII<br>carboxyl end of IC3<br>TMV | 128<br>AVDVLCCTASI SEQ ID NO: 9<br>F<br>293<br>REKKAAKTLGI SEQ ID NO: 10<br>E<br>204<br>EEPFYALFSSLG SEQ ID NO: 11<br>V | IP / COS-1<br>IP<br>arachidonic acid release<br>IP / COS-1 | (Perez, Hwa et al. 1996)<br>(Hwa, Gaivin et al. 1997) |
| A1AB_human | $\alpha_{1B}$-adrenergic | C-terminal IC3 | 293<br>SREKKAAKT SEQ ID NO: 12<br>X=19 different substitutions | PI / COS-7 | (Kjelsberg, Cotecchia et al. 1992) |
| A1AB_human | $\alpha_{1B}$-adrenergic | C-terminus IC3 | 288 293<br>KFSREKKAAKTLGI SEQ ID NO: 13<br>K H L | PI hydrolysis / rat fibroblast | (Allen, Lefkowitz et al. 1991) |
| A2AA_human | $\alpha_2$C10-adrenergic<br>alpha-2AAR | C-terminal IC3 loop | 373 (348?)<br>EKRFTFVLAV SEQ ID NO: 14<br>X=F,A,C,E,K | adenylyl cyclase inhibition / HEK293 | (Ren, Kurose et al. 1993) |
| ACM1_human | muscarinic Hm1<br>muscarinic acetylcholine M1 | C-terminal IC3 loop junction | 360<br>SLVKEKKAARTLS SEQ ID NO: 15<br>A | PI / HEK(U293) | (Högger, Shockley et al. 1995) |
| ACM2-human | muscarinic acetylcholine M2 | junction of IC3 and TMVI | 390<br>KKVTRTIL↑A SEQ ID NO: 16<br>1-4 A inserted | IP production, inhibition of cAMP production / COS-7 | (Liu, Blin et al. 1996) |

FIG. 1-3

| CLASS A GROUP II | | | | | |
|---|---|---|---|---|---|
| ACM3_rat | m3 muscarinic (rat)<br>muscarinic acetylcholine M3 | TMVI | 507<br>TWTPYNIMVLVNT SEQ ID NO: 17<br>S | IP / COS-7 | (Blüml, Mutschler et al. 1994) |
| ACM5_human | m5 muscarinic<br>muscarinic acetylcholine M5 | N-terminus to TMII<br>TMVI | chimera composed of<br>m2 1-69<br>m5 77-445<br>m2 391-466 | β-gal / NIH 3T3 | (Burstein, Spalding et al. 1996) |
| ACM5_human | m5 muscarinic<br>muscarinic acetylcholine M5 | TMVI<br>SEQ ID NO: 18 | 451 459 465<br>AILLA FIITW TPYNI MVLVST<br>M L H C<br>V S F<br>T | β-gal; radioligand binding / NIH-3T3 | (Spalding, Burstein et al. 1998) |
| ACM5_human | m5 muscarinic<br>muscarinic acetylcholine M5 | junction of TMVI and EC3 | 465<br>YNIMVLVSTFCDKCV SEQ ID NO: 19<br>X=V,F,R,K,+more | β-gal; radioligand binding / NIH-3T3 | (Spalding, Burstein et al. 1997) |
| B1AR_human | $\beta_1$-adrenergic | C-terminus | 389<br>RKAFQGLLCCA SEQ ID NO: 20<br>R | adenylyl cyclase; agonist binding / CHW | (Mason, Moore et al. 1999) |
| B2AR_human | $\beta_2$-adrenergic<br>beta-2AR | C-terminal IC3 loop | 266 272<br>FCLKEHKALKTLGI SEQ ID NO: 21<br>SR K A | adenylyl cyclase activation; agonist binding affinity / COS-7 or CHO | (Samama, Cotecchia et al. 1993);<br>(Lefkowitz, Cotecchia et al. 1993) |
| DADR_human | dopamine<br>D1A | carboxyl terminal IC3 | 264<br>SFKMSFKRETKVLKT SEQ ID NO: 22<br>I K<br>288 *from D1B receptor*<br>APDTSIKRETKVLKT SEQ ID NO: 23 | adenylyl cyclase; cAMP accumulation / HEK293 | (Charpentier, Jarvie et al. 1996) |
| DADR_human | dopamine<br>D1 | TMVI | 286<br>FVCCWLPFFIL SEQ ID NO: 24<br>A | cAMP accumulation / COS-7 | (Cho, Taylor et al. 1996) |
| HH2R_rat | histamine $H_2$ | IC2 | 115<br>FMISLDRYCAV SEQ ID NO: 25<br>N,A | cAMP production / HEK-293 | (Alewijnse, Timmerman et al. 2000) |

FIG. 1-4

| File Name | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CLASS A GROUP III | | | | | |
| OPSD_human | opsin<br>rhodopsin | TMII<br>TMIII<br>TMVII | 90<br>FMVLGGFTSTLY SEQ ID NO: 26<br>D<br>113<br>GCNLEGFFAT SEQ ID NO: 27<br>Q<br>292 296<br>MTIPAFFAKSAAIY SEQ ID NO: 28<br>E G,E,M<br>$^{292}$Ala neutral a.a converted to carboxylate and competes with $^{113}$Glu for salt bridge with $^{296}$Lys | transducin; phosphorylation by rhodopsin kinase / COS | (Rim and Oprian 1995) |
| OPSD_human | opsin<br>rhodopsin | TMIII | 134<br>VVLAIERYVVV SEQ ID NO: 29<br>I,Q,S | transducin; radioligand binding / COS | (Acharya and Karnik 1996) |
| OPSD_human | opsin<br>rhodopsin | TM6<br>*plus* TM3 | 257<br>RMVIIMVIAFL SEQ ID NO: 30<br>Y,N<br>*plus* G113Q | transducin, GTPγS uptake / COS | (Han, Smith et al. 1998) |
| OPSD_human | opsin<br>rhodopsin | TMVII<br>IC2 | 296<br>PAFFAKSAAIY SEQ ID NO: 31<br>G<br>X=E,M natural mutants<br>+ 10 different a.a. substitutions<br>disrupts critical salt bridge between $^{296}$Lys(TMVII) and $^{113}$Glu(TMIII)<br>134<br>VVLAIERYVVV SEQ ID NO: 32<br>Q | transducin; radioligand binding / COS | (Govardhan and Oprian 1994);<br>(Cohen, Yang et al. 1993)<br>(Cohen, Yang et al. 1993) |

FIG. 1-5

| | | | | | |
|---|---|---|---|---|---|
| TRFR_mouse | thyrotropin-releasing hormone TRH-R | carboxyl tail | 335<br>FRKLCNCKQK SEQ ID NO: 33<br>STOP | $^{45}Ca^{2+}$ efflux, $[Ca^{2+}]$ / Xenopus oocytes; IP formation / AtT20, *stably transfected* | (Matus-Leibovitch, Nussenzveig et al. 1995) |

FIG. 1-6



| File Name | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CLASS A GROUP IV | | | | | |
| BRB2_human | bradykinin $B_2$<br><br>B2 bradykinin<br>BK-2 | TMIII<br><br>TMVI | 113<br>AIISMNLYSSI SEQ ID NO: 34<br>A<br>256<br>LLFIICWLPFQI SEQ ID NO: 35<br>F | IP production / COS-7 | (Marie, Koch et al. 1999) |

FIG. 1-7

| File Name | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CLASS A GROUP V | | | | | |
| AG2R_rat | $AT_{1A}$<br>Type-1A angiotensis II | TMIII | 111<br>ASVSFNLYASV SEQ ID NO: 36<br>A<br>disrupts $^{111}$Asn(TMIII) - $^{292}$Tyr(TMVII) interaction | phospholipase C; IP production / COS-7 | (Groblewski, Maigret et al. 1997) |
| AG2R_rat | $AT_{1A}$<br>Type-1A angiotensis II | C-terminus of TM7<br>other multiple mutations | 305<br>LFYGFLGKKFK SEQ ID NO: 37<br>Q | IP production / HEK-293; intrcellular $Ca^{2+}$ mobilization / CHO | (Parnot, Bardin et al. 2000) |
| FMLR_human | formylmethionylleucylphenylalanine (fMLPR) | IC1<br>SEQ ID NO: 38<br>SEQ ID NO: 39 | 51<br>LVIWVAGFRMTHTVTTISYLNKAVA<br>LVVWVTAFEAKRTINAIWFLNLAVA<br>(K above conflicts with SWISS-PROT database) | PI production; phospholipase C stimulation / COS-7 | (Amatruda, Dragas-Graonic et al. 1995) |
| IL8B_human | interleukin-8 receptor B<br>CXCR-2 chemokine | IC2 | 138<br>ACISVDRYLAIVH SEQ ID NO: 40<br>V | IP production; $Ca^{2+}$ moblization and actin polymerization / NIH 3T3 | (Burger, Burger et al. 1999) |
| LSHR_human | luteinizing hormone (LH) | IC3 | 564<br>MATNKDTKIAKK SEQ ID NO: 41<br>G | cAMP production / HEK293 | (Kudo, Osuga et al. 1996) |
| LSHR_human | luteinizing hormone (LH) | TMVI | 578<br>ILIFTDFTCMA SEQ ID NO: 42<br>G | cAMP production / COS-7 | (Shenker, Laue et al. 1993) |
| LSHR_human | luteinizing hormone (LH) | TM6<br>SEQ ID NO: 43 | 571 577<br>KIAKKMAILIFTDFTCM<br>I I | cAMP production / COS-7 | (Kosugi, Van Dop et al. 1995) |
| LSHR_rat | luteinizing hormone / human chorionic gonadotropin (LH/hCG) | TMVI | 556<br>ILIFTDFTCMA SEQ ID NO: 44<br>G, Y | cAMP production / HEK 293T | (Bradbury, Kawate et al. 1997; Bradbury and Menon 1999) |
| OPRD_mouse | delta opiod receptor | TM3 | 128<br>KVLSIDYYNMF SEQ ID NO: 45<br>A, K, H | adenylyl cyclase inhibition / COS-7 | (Cavalli, Babey et al. 1999) |
| OXYR_human | oxytocin | IC2 | 137<br>LMSLDRCLAIC SEQ ID NO: 46<br>A | IP production / COS-7 | (Fanelli, Barbier et al. 1999) |

FIG. 1-8

| | | | | | |
|---|---|---|---|---|---|
| PAFR_human | platelet-activating factor (PAF) | C-terminus of IC3 | 231<br>EVKRRALWMVCTVLAV SEQ ID NO: 47<br>R | IP production / COS-7 | (Parent, Le Gouill et al. 1996) |
| PAFR_human | platelet-activating factor (PAF) | TMIII | 100<br>CLFFINTYCSV SEQ ID NO: 48<br>A | arachnidonate release, IP production, adenylyl cylcase inhibition / CHO | (Ishii, Izumi et al. 1997) |
| PE23_human | prostaglandin $E_2$,<br>EP3III<br>EP3IV | C-terminal tail | 360<br>FCQEEFWGN SEQ ID NO: 49<br>FCQMRKRRLREQEEFWGN SEQ ID NO: 50<br>↑truncated | inhibition of adenylyl cyclase / CHO-K1 | (Jin, Mao et al. 1997) |
| PE23_mouse | prostaglandin $E_2$<br>EP3 | carboxyl-terminal tail<br>SEQ ID NO: 51 | 336<br>KILLRKFCQIRDHT (3α)<br>MMNHL (3β)<br>↑truncated | inhibition of adenylate cyclase / CHO, *stably expressed* | (Hasegawa, Negishi et al. 1996) |
| THRR_human | thrombin | EC2 loop<br>SEQ ID NO: 52 | 259 268<br>CHDVLNETLLEGYYAYY<br>DLKD KDF I | $^{45}Ca^{2+}$ efflux, PI hydrolysis, reporter gene induction / COS-7 | (Nanevicz, Wang et al. 1996) |
| TSHR_human | thyrotropin (TSHR)<br>thyroid stimulating hormone | EC1 | 486<br>YYNHAIDWQTG SEQ ID NO: 53<br>F, M | inositol phosphate--diacylglycerol cascade / COS-7 | (Parma, Van Sande et al. 1995) |
| | | EC2 | 568<br>YAKVSICLPMD SEQ ID NO: 54<br>T | | |
| TSHR_human | thyrotropin (TSHR)<br>thyroid stimulating hormone | TMIII | 509<br>ASELSVYTLTV SEQ ID NO: 55<br>A | adenylyl cyclase activation / COS-7 | (Duprez, Parma et al. 1994) |
| | | TMVII | 672<br>YPLNSCANPFL SEQ ID NO: 56<br>Y | | |
| TSHR_human | thyrotropin (TSHR)<br>thyroid stimulating hormone | TMV | 597<br>VAFVIVCCCHV SEQ ID NO: 57<br>L | cAMP formation / COS-7 cells | (Esapa, Duprez et al. 1999) |
| TSHR_human | thyrotropin (TSHR)<br>thyroid stimulating hormone | TMVII | 677<br>CANPFLYAIFT SEQ ID NO: 58<br>V | cAMP formation / CHO cells | (Russo, Wong et al. 1999) |
| TSHR_human | thyrotropin (TSHR)<br>thyroid stimulating hormone | IC3 | 613 621<br>VRNPQYNPGDKDTKIAK<br>deletion SEQ ID NO: 59 | cAMP formation / COS-7 | (Wonerow, Schoneberg et al. 1998) |

FIG. 1-9



| | | | | | |
|---|---|---|---|---|---|
| TSHR_human | thyrotropin (TSHR)<br><br>thyroid stimulating hormone | IC3 / TMVI<br>SEQ ID NO: 60 | 623 632<br>KDTKIAKRMAVLIFTDFICM<br>V I | cAMP activation / COS-7 | (Paschke, Tonacchera et al. 1994) |
| V2R_human | vasopressin V2 | IC2<br>SEQ ID NO: 61 | 136<br>LAMTLDRHRAI<br>A | cAMP formation / COS-7 | (Morin, Cotte et al. 1998) |

FIG. 1-10

| File Name | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CLASS B GROUP I | | | | | |
| CALR_human | human calcitonin hCTR-1 hCTR-2 | wild type (native) protein | | adenylyl cyclase cAMP production / COS-1 | (Cohen, Thaw et al. 1997) |
| CLASS B GROUP II | | | | | |
| PTRR_human | parathyroid hormone PTH / PTH-related peptide | junction of IC1 and TMII<br><br>junction of IC3 and TMVI | 223<br>TRNYIHMHLFL SEQ ID NO: 62<br>R,K<br><br>410<br>KLLKSTLVLMP SEQ ID NO: 63<br>C,others | cAMP accumulation / COS-7 | (Schipani, Jensen et al. 1997) |
| | | | | | |
| | | | | | |
| CLASS B GROUP III | | | | | |
| GIPR_human | glucose-dependent insulinotropic peptide (GIP-R) | TMVI | 340<br>VFAPVTEEQAR SEQ ID NO: 64<br>P | cAMP production / L293 | (Tseng and Lin 1997) |
| GLR_rat | glucagon | junction of IC loop1 and TMII<br><br>IC end of TMVI | 178<br>TRNYIHGNLFA SEQ ID NO: 65<br>R<br><br>352<br>RLARSTLTLIP SEQ ID NO: 66<br>A | cAMP accumulation / COS-7 | (Hjorth, Orskov et al. 1998) |
| VIPR_human | vasoactive intestinal peptide 1 (VIP) | junction of IC loop 1 and TMII<br><br>junction of IC loop 3 and TMVI | 178<br>RNYIHMHLFI SEQ ID NO: 67<br>R *requires functional integrity of the N-terminal EC domain*<br><br>343<br>LARSTLLLIP SEQ ID NO: 68<br>X= K,P | cAMP production / COS-7 or CHO | (Gaudin, Maoret et al. 1998)<br>(Gaudin, Rouyer-Fessard et al. 1998) |

FIG. 1-11



| File Name<br>CLASS C | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CASR_human | calcium-sensing | N-terminal EC | TLSFVAQNKIDSLNLDEFCNCSEHI<br>*various substitutions, in multiple combinations* SEQ ID NO: 69 | IP / tsA | (Jensen, Spalding et al. 2000) |
| | | | | | |

FIG. 1-12

| File Name | Receptor | Mutation Site | Sequence | Assay / Cells | Reference |
|---|---|---|---|---|---|
| CLASS D | | | | | |
| O74283<br>RCB2<br>C. cinereus | pheromone | TM6 | 229<br>PLSAYQIYLGT SEQ ID NO: 70<br>P | heterologous yeast assay | (Olesnicky, Brown et al. 1999) |
| STE2_yeast | pheromone α-factor | TM6 | 258<br>QSLLVPSIIFI SEQ ID NO: 71<br>LL | *lacZ* reporter gene | (Konopka, Margarit et al. 1996) |
| STE2_yeast | pheromone α-factor | double mutations TM5<br><br>and<br><br>TM6 | 223<br>MSFVLVVKHILAIR SEQ ID NO: 72<br>C C<br>247 251<br>DSFHILLIMSCQSLL SEQ ID NO: 73<br>CC CC<br><br>*double mutations*<br>shaded *double mutations* | *lacZ* reporter gene / yeast | (Dube, DeCostanzo et al. 2000) |
| STE3_yeast | pheromone a-factor | IC3 | 194<br>DVRDILHCTNS SEQ ID NO: 74<br>Q | β-galactosidase | (Boone, Davis et al. 1993) |
| STE2_yeast | pheromone α-factor | TM6 | 253 258<br>LIMSCQSLLVPSIIFI SEQ ID NO: 75<br>L LP | β-galactosidase | (Sommers, Martin et al. 2000) |

# FIG. 1-13

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# FIG. 1-14


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# FIG. 1-15


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FIG. 2

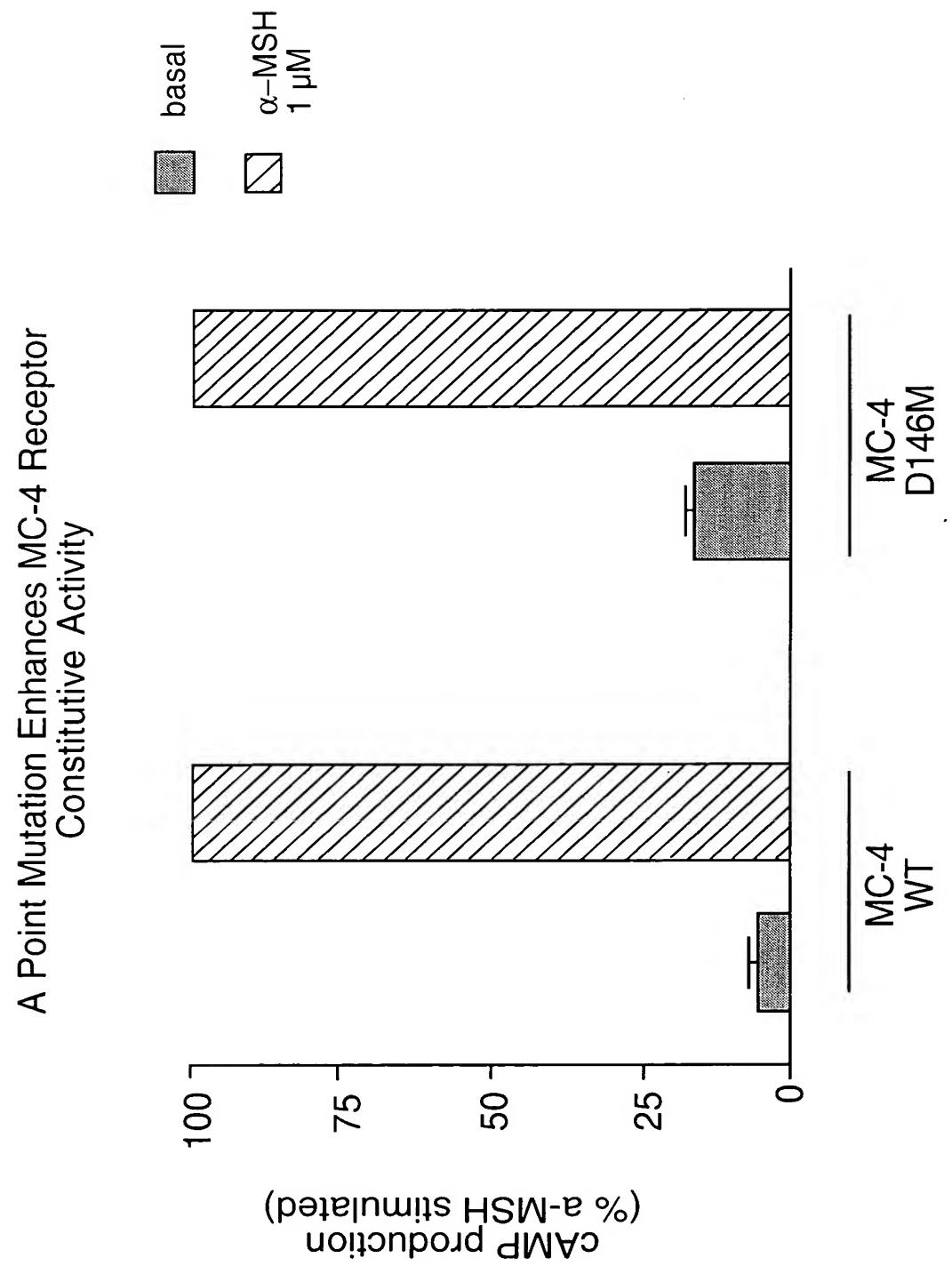

A Point Mutation Enhances MC-4 Receptor Constitutive Activity
basal
α–MSH 1 μM
cAMP production (% a-MSH stimulated)
100
75
50
25
0
MC-4 WT
MC-4 D146M

FIG. 3

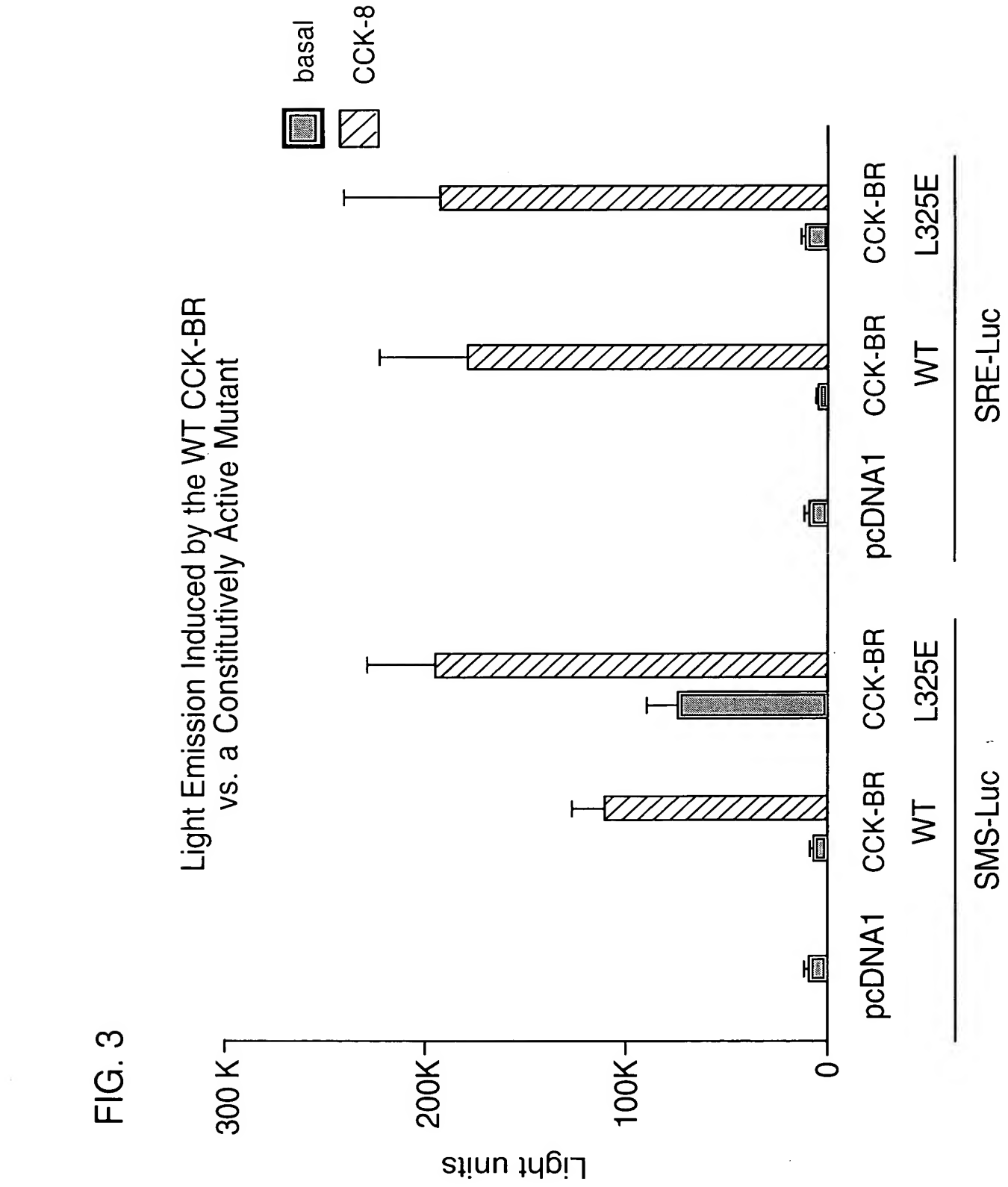

Light Emission Induced by the WT CCK-BR
vs. a Constitutively Active Mutant
basal
CCK-8
Light units
300 K
200K
100K
0
pcDNA1
CCK-BR
WT
CCK-BR
L325E
SMS-Luc
pcDNA1
CCK-BR
WT
CCK-BR
L325E
SRE-Luc

FIG. 4

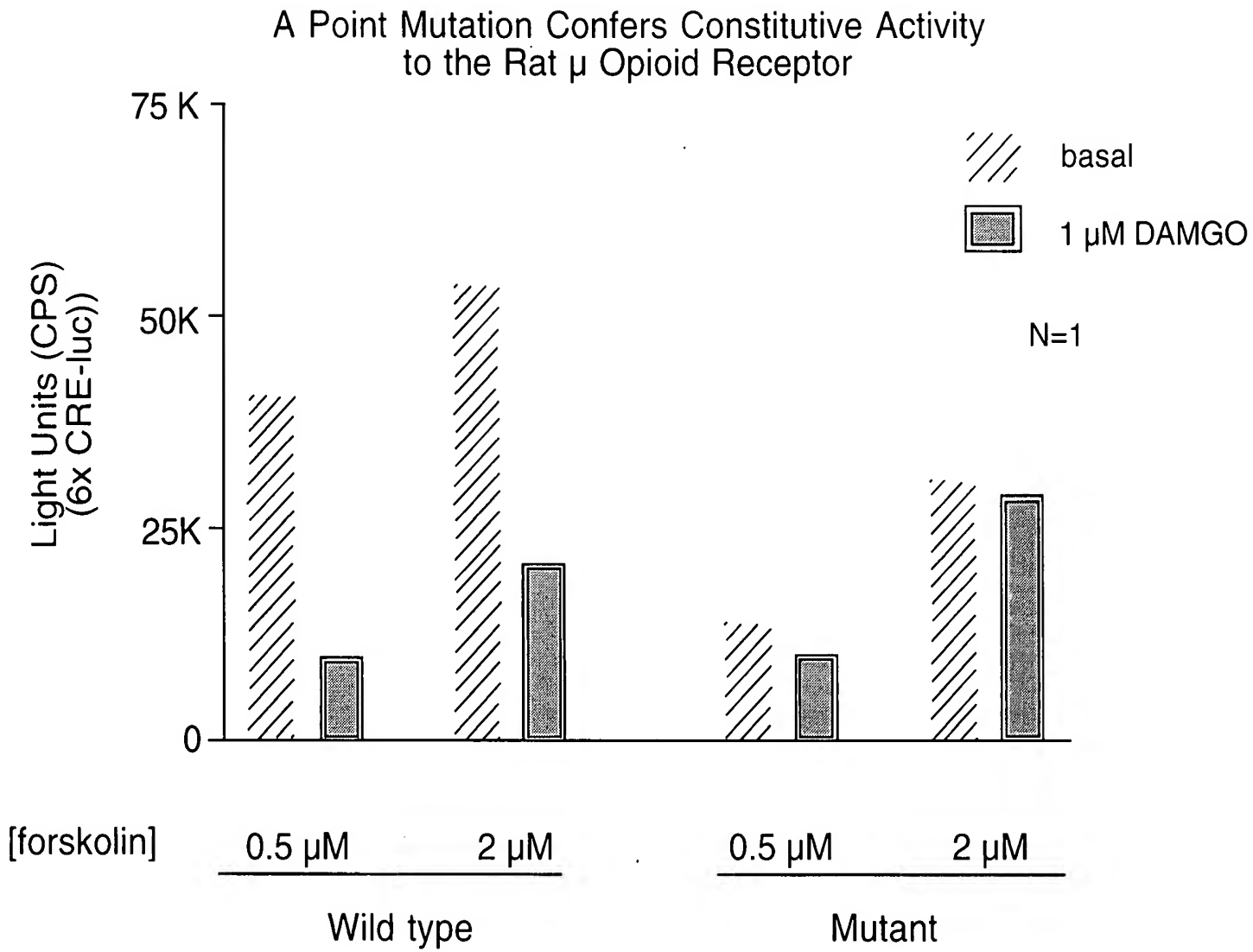


FIG. 5

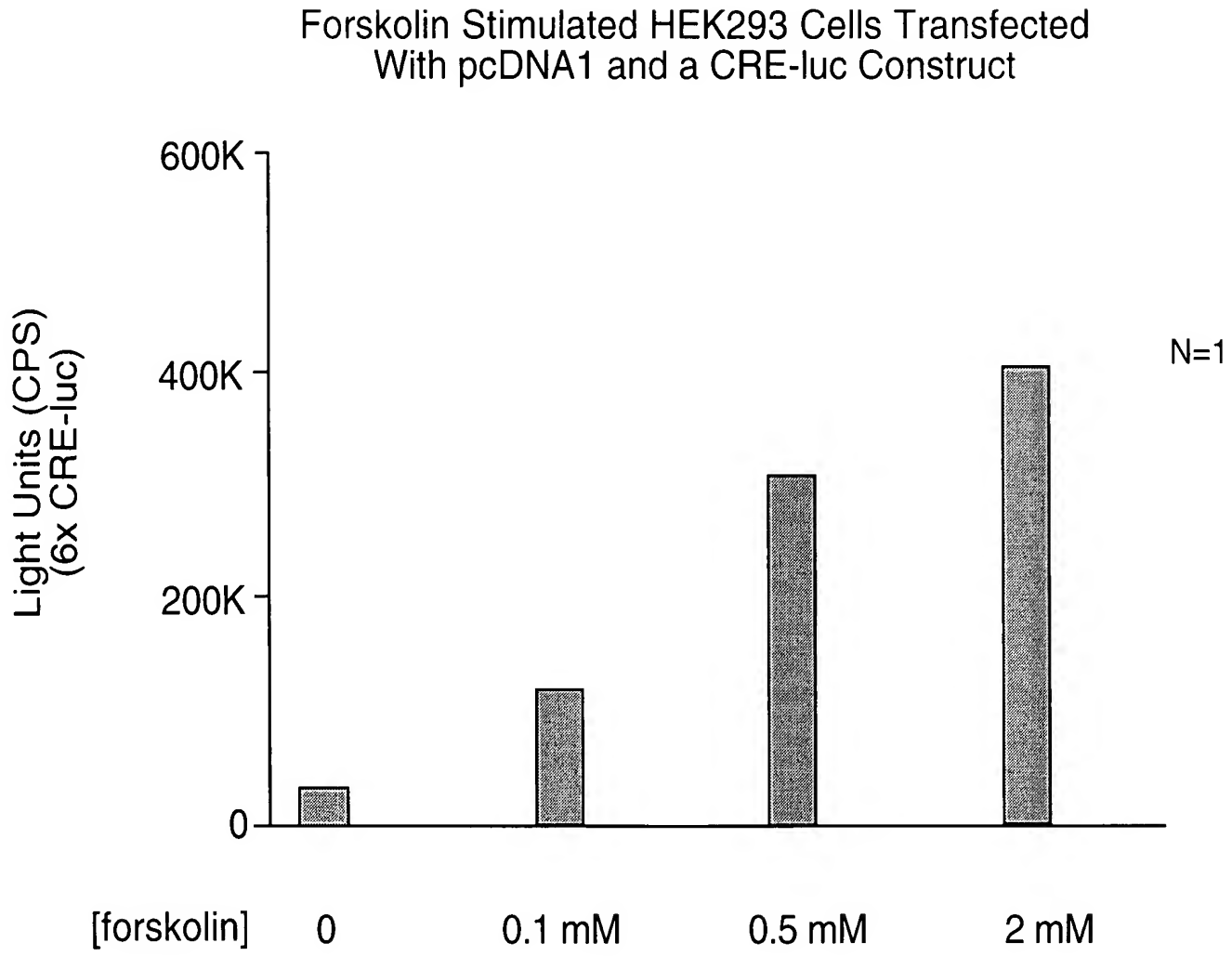


FIG. 6

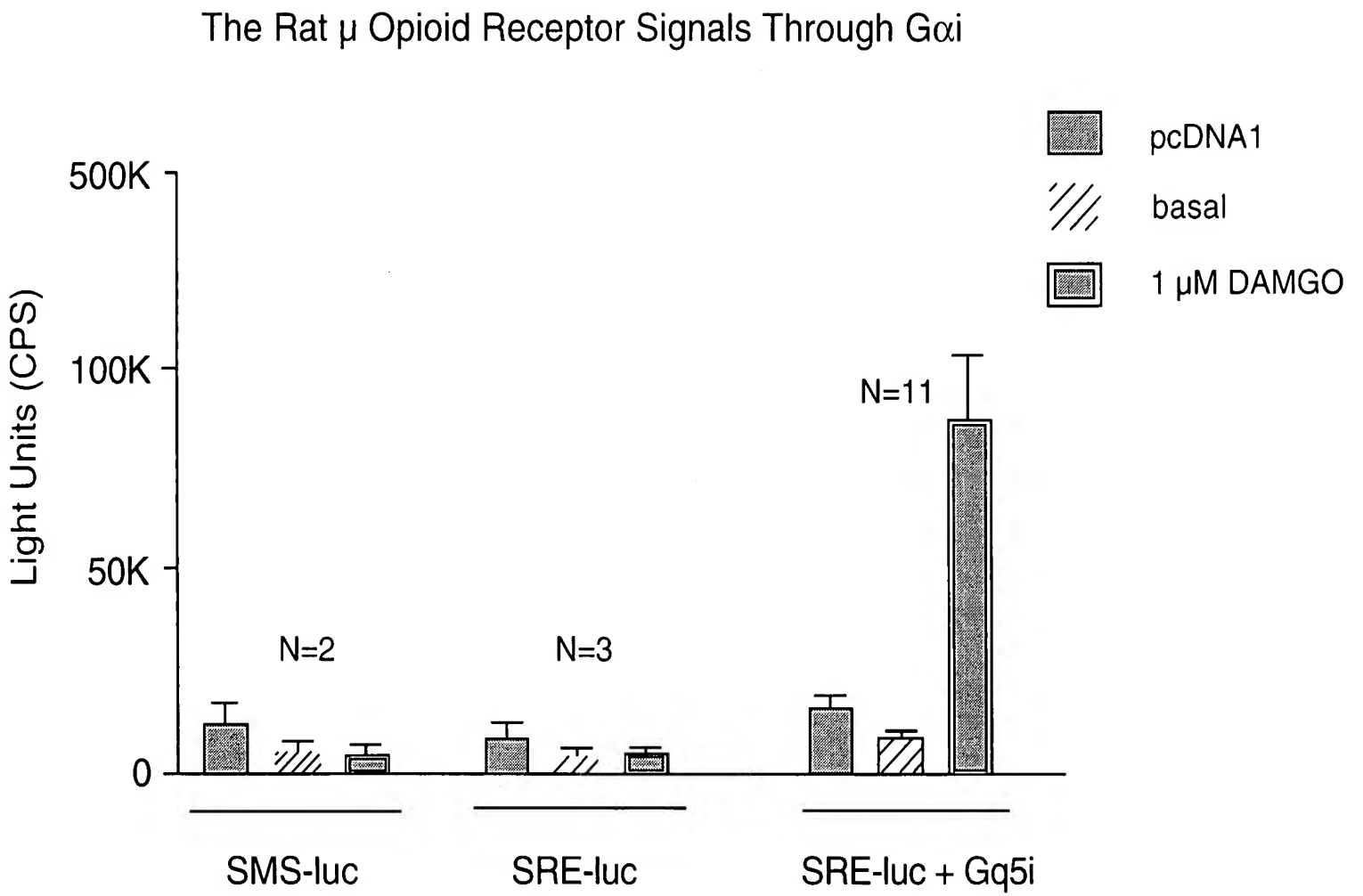


FIG. 7

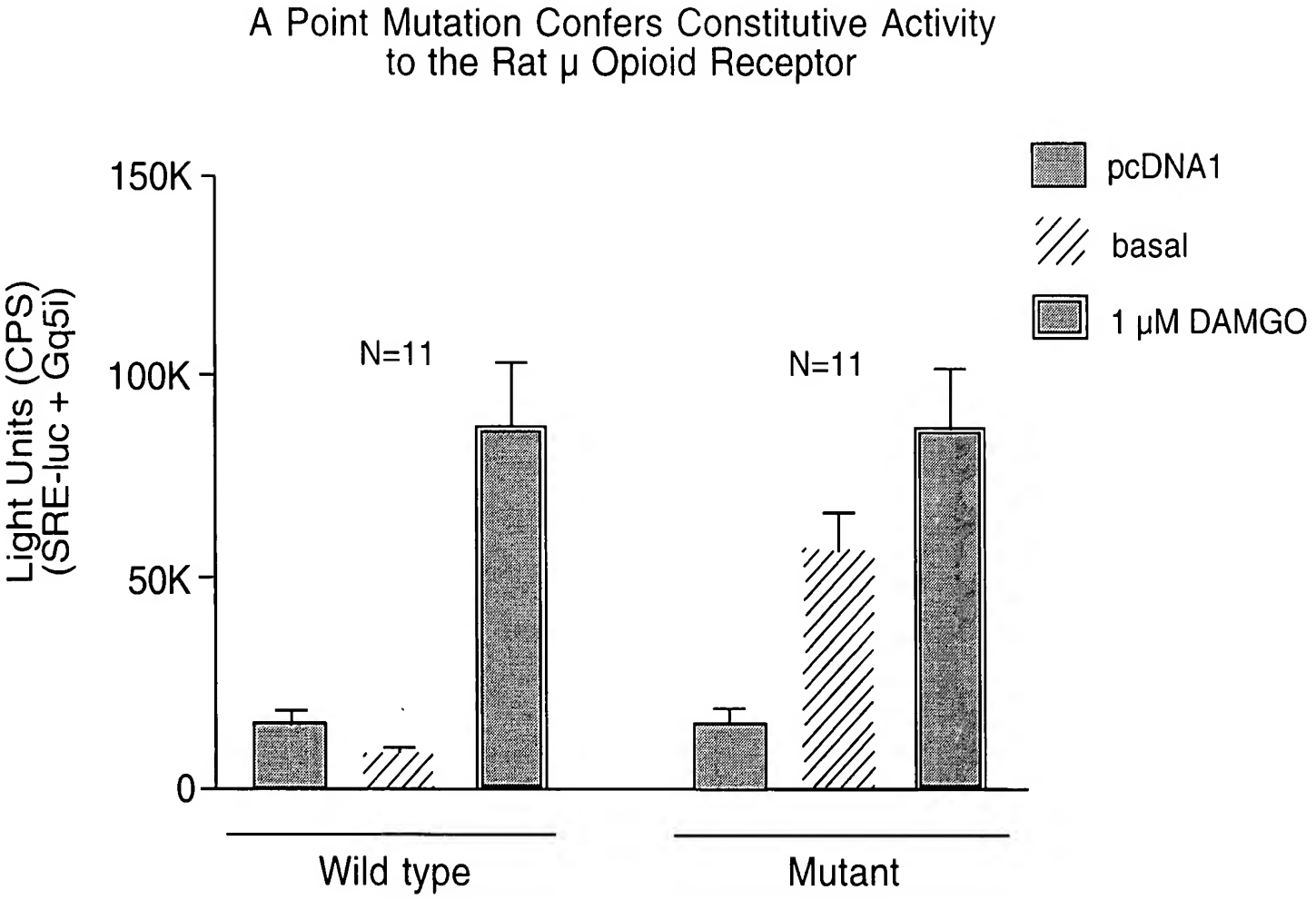


FIG. 8

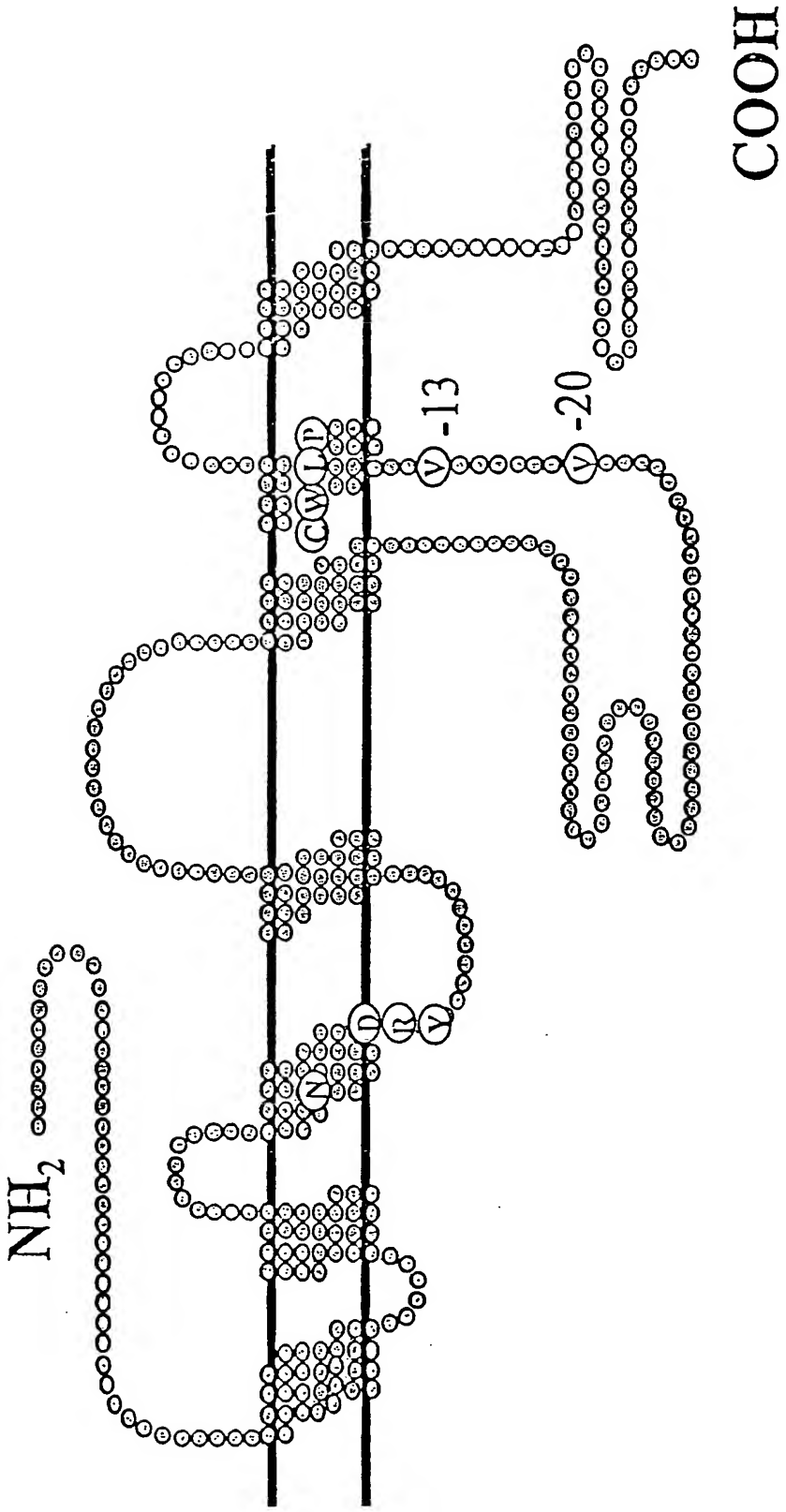


FIG. 9

TMD III Asn (-14 from DRY) is a Target
for Mutation Induced Constitutive Activity

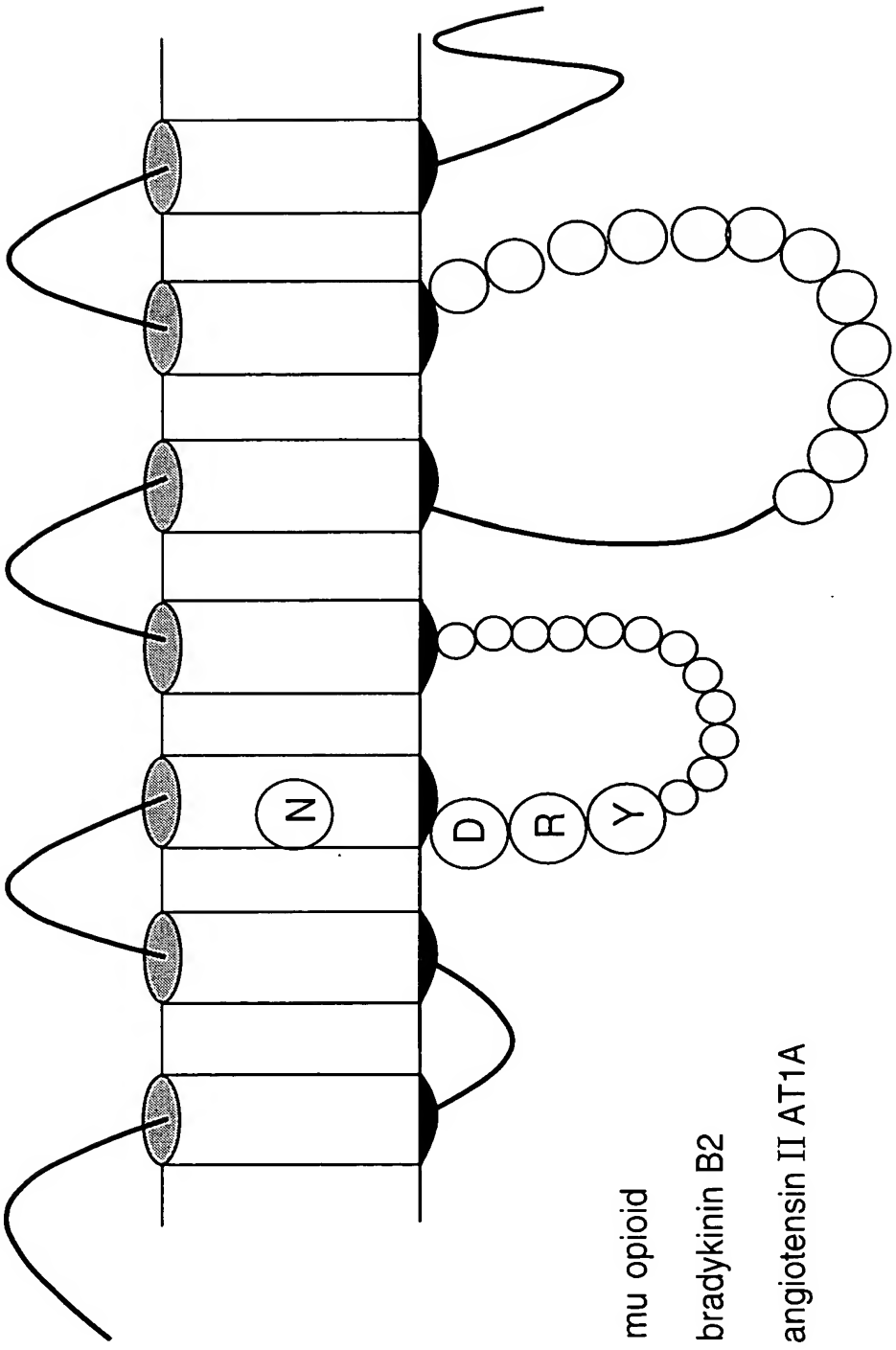
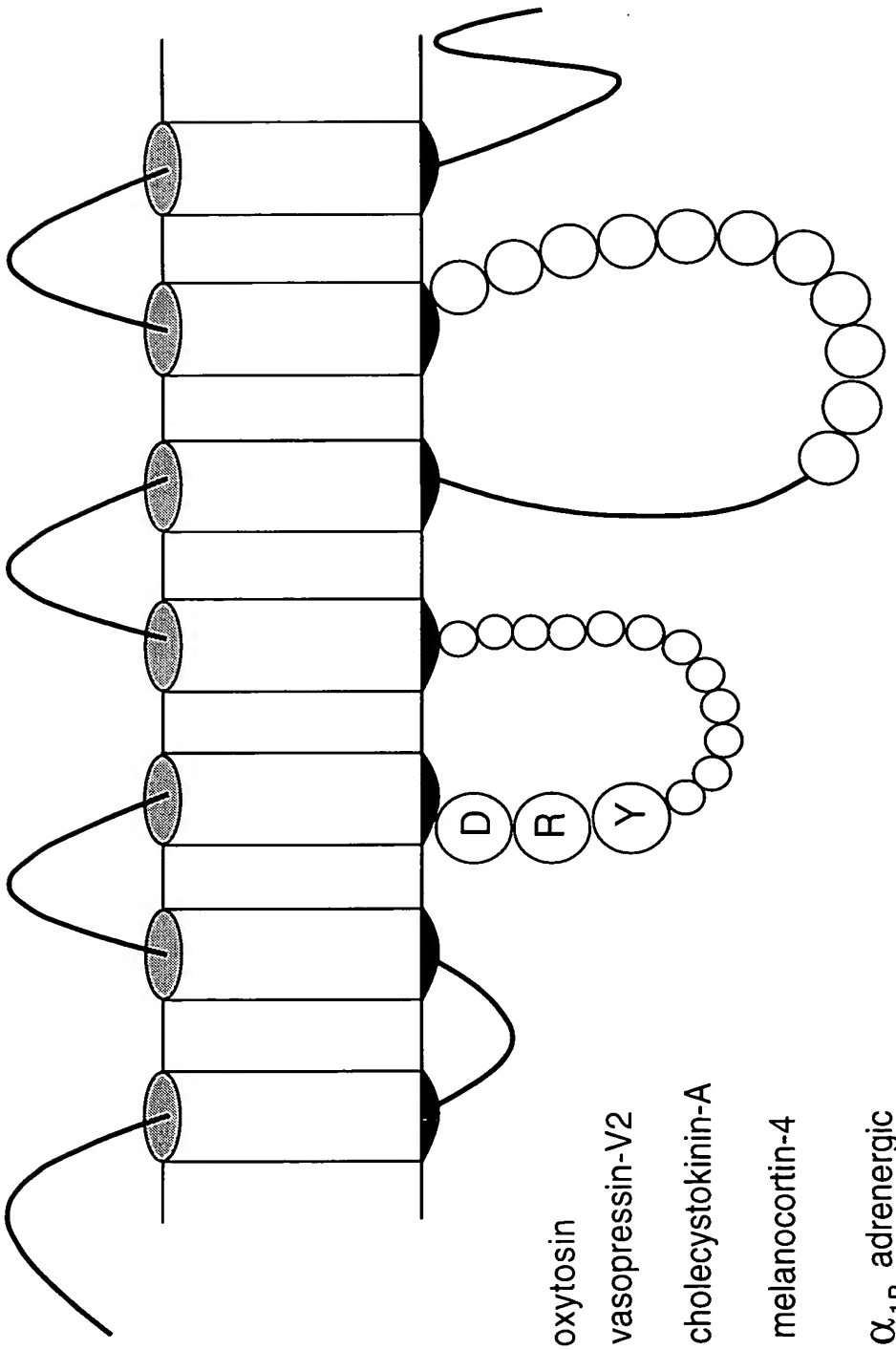


FIG. 10

The 'DRY' Motif is a Target for Mutation Induced Constitutive Activity




oxytosin

vasopressin-V2

cholecystokinin-A

melanocortin-4

$\alpha_{1B}$ adrenergic

FIG. 11

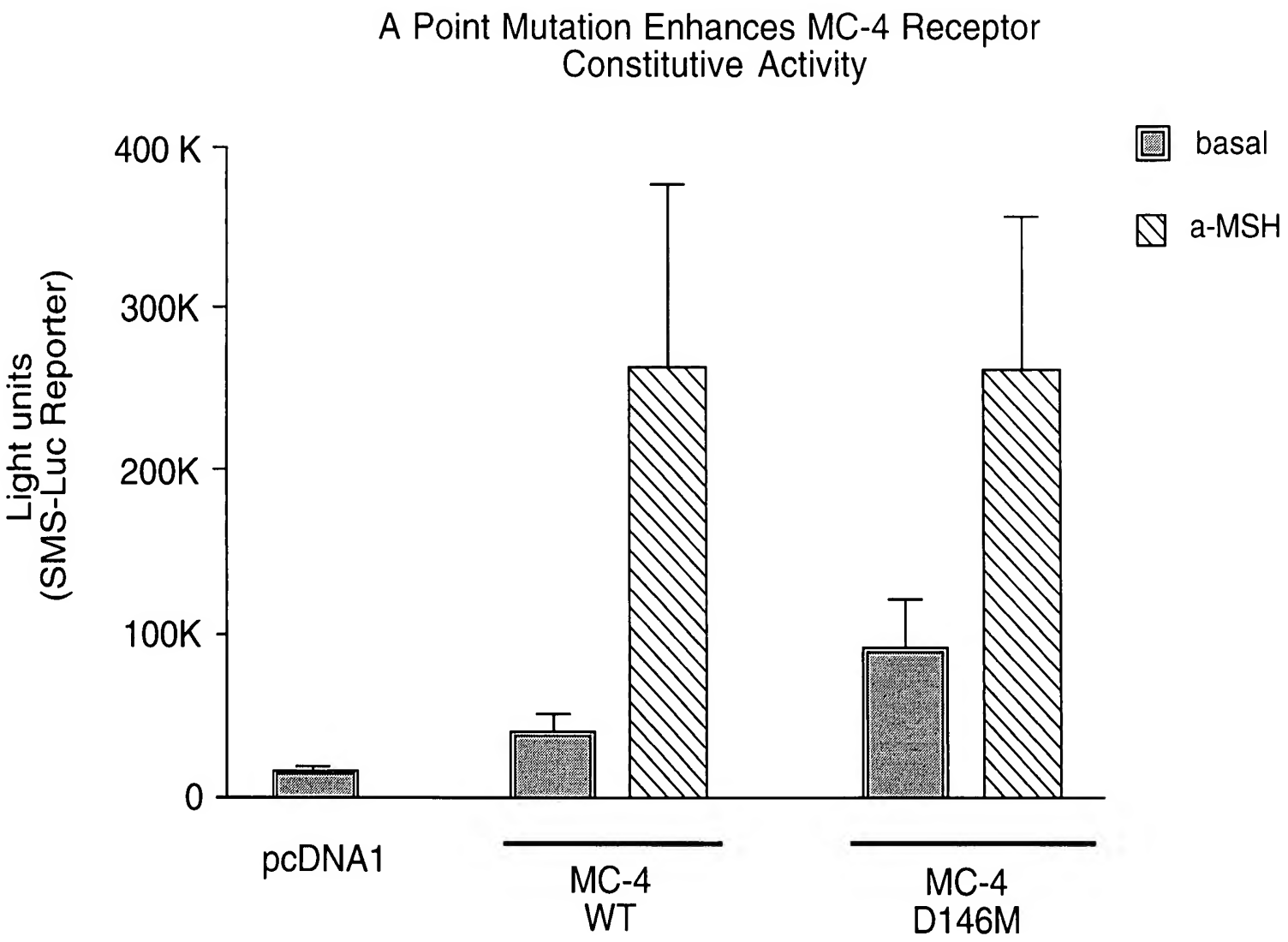
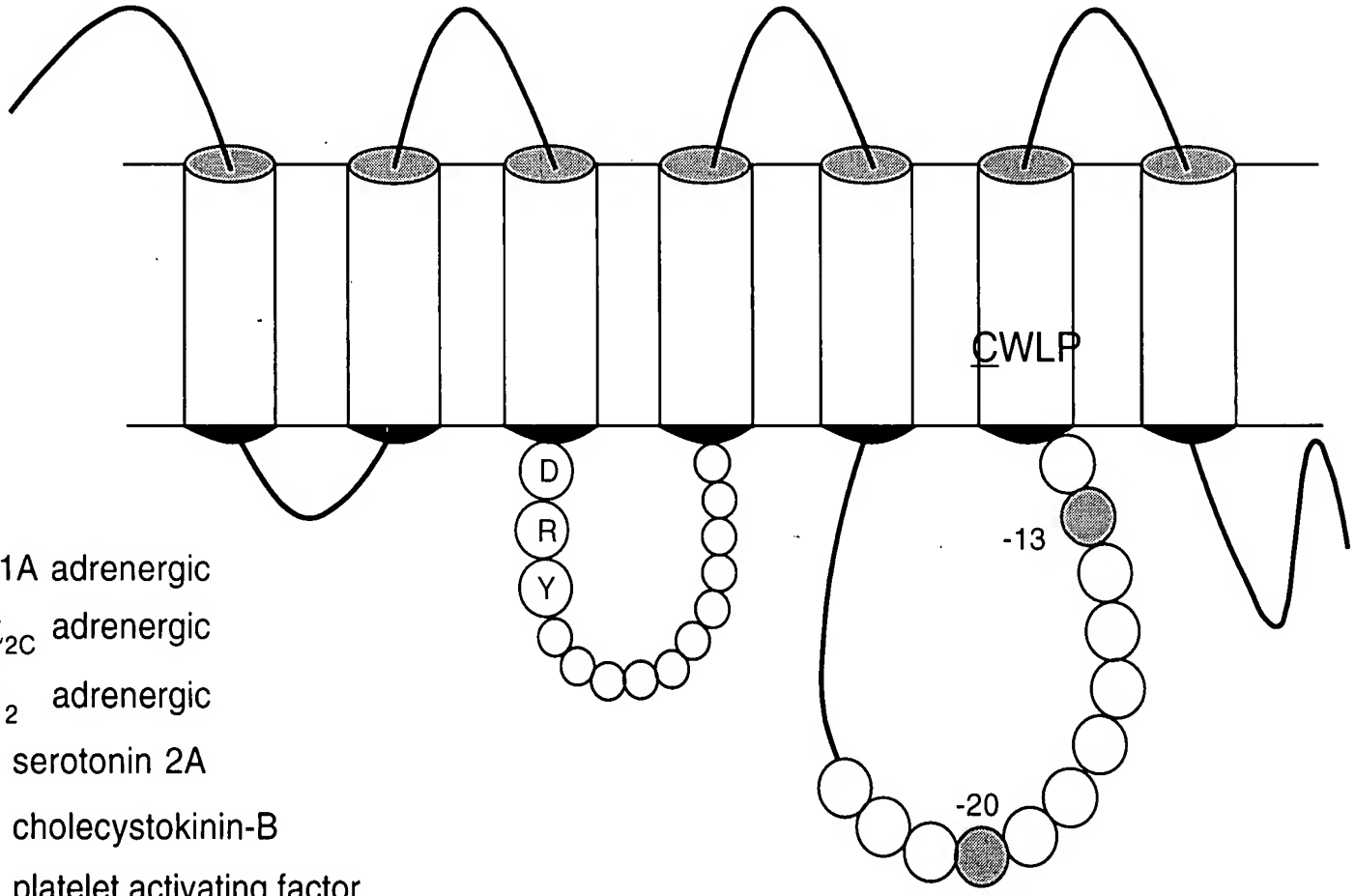


FIG. 12

The -13 Position is a Target for Mutation
Induced Constitutive Activity




1A adrenergic

$\alpha_{2C}$ adrenergic

$\beta_2$ adrenergic

serotonin 2A

cholecystokinin-B

platelet activating factor

thyroid stimulating hormone

FIG. 13

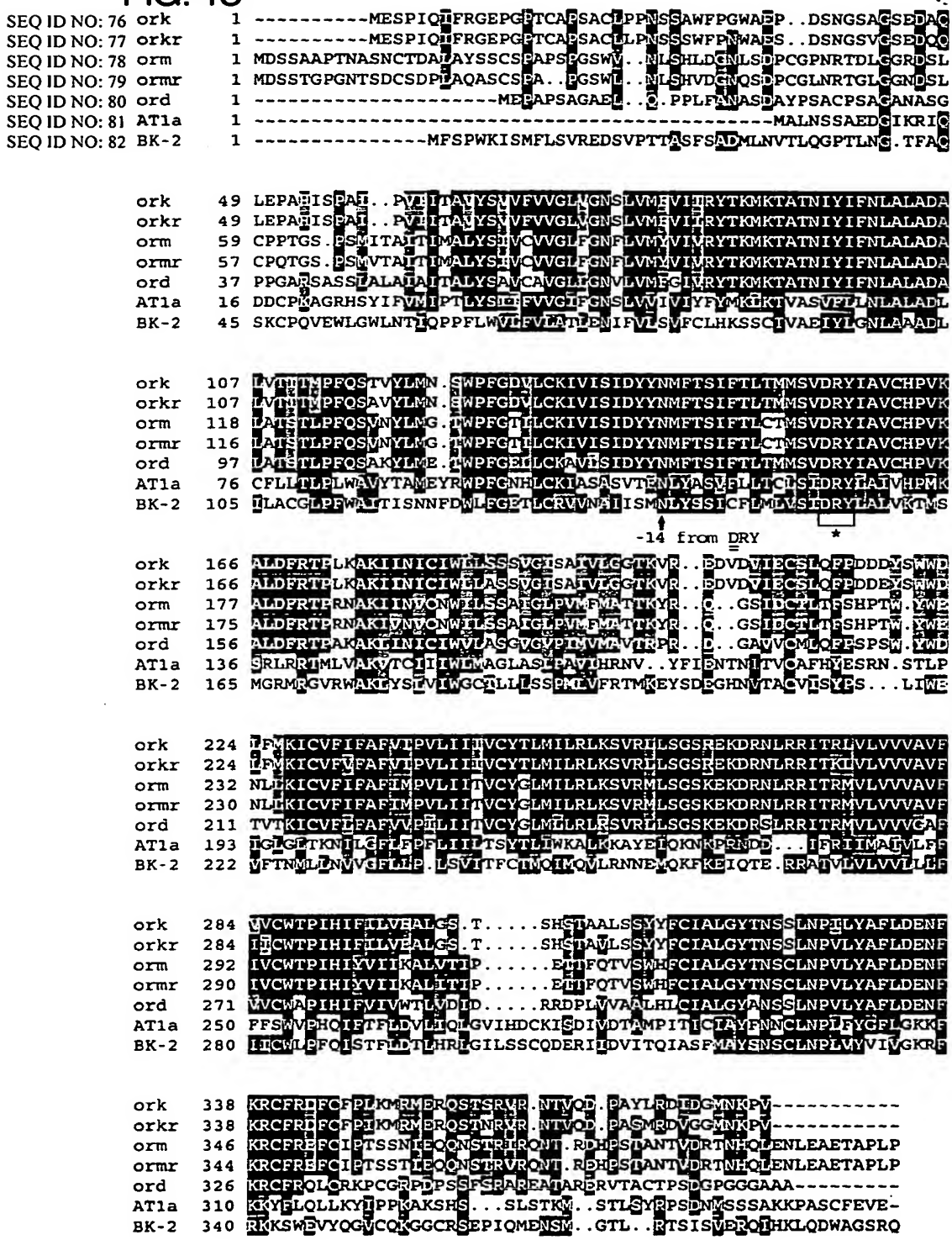

SEQ ID NO: 76 ork
SEQ ID NO: 77 orkr
SEQ ID NO: 78 orm
SEQ ID NO: 79 ormr
SEQ ID NO: 80 ord
SEQ ID NO: 81 AT1a
SEQ ID NO: 82 BK-2
-14 from DRY

FIG. 14

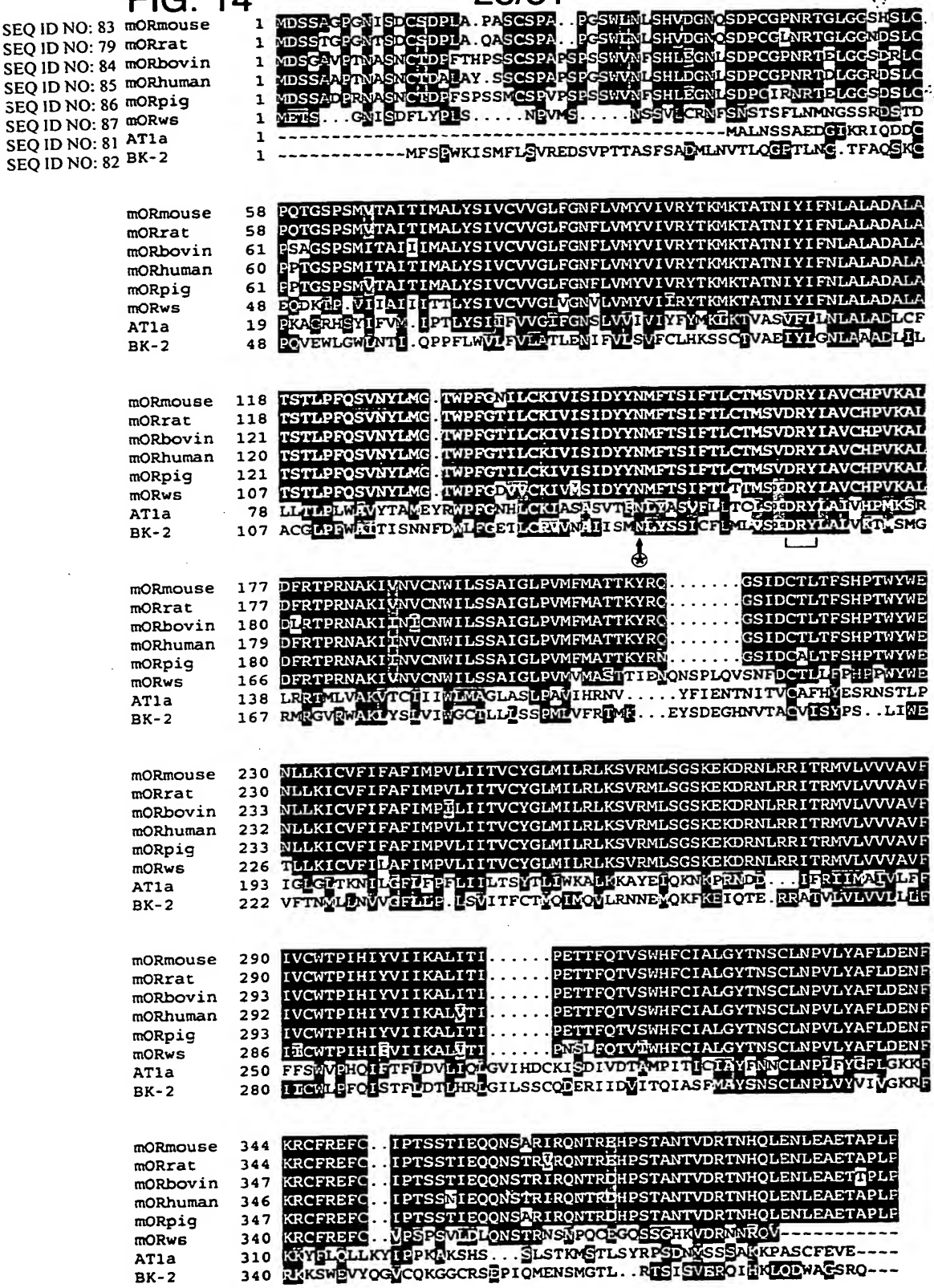

SEQ ID NO: 83 mORmouse
SEQ ID NO: 79 mORrat
SEQ ID NO: 84 mORbovin
SEQ ID NO: 85 mORhuman
SEQ ID NO: 86 mORpig
SEQ ID NO: 87 mORws
SEQ ID NO: 81 AT1a
SEQ ID NO: 82 BK-2

FIG. 15

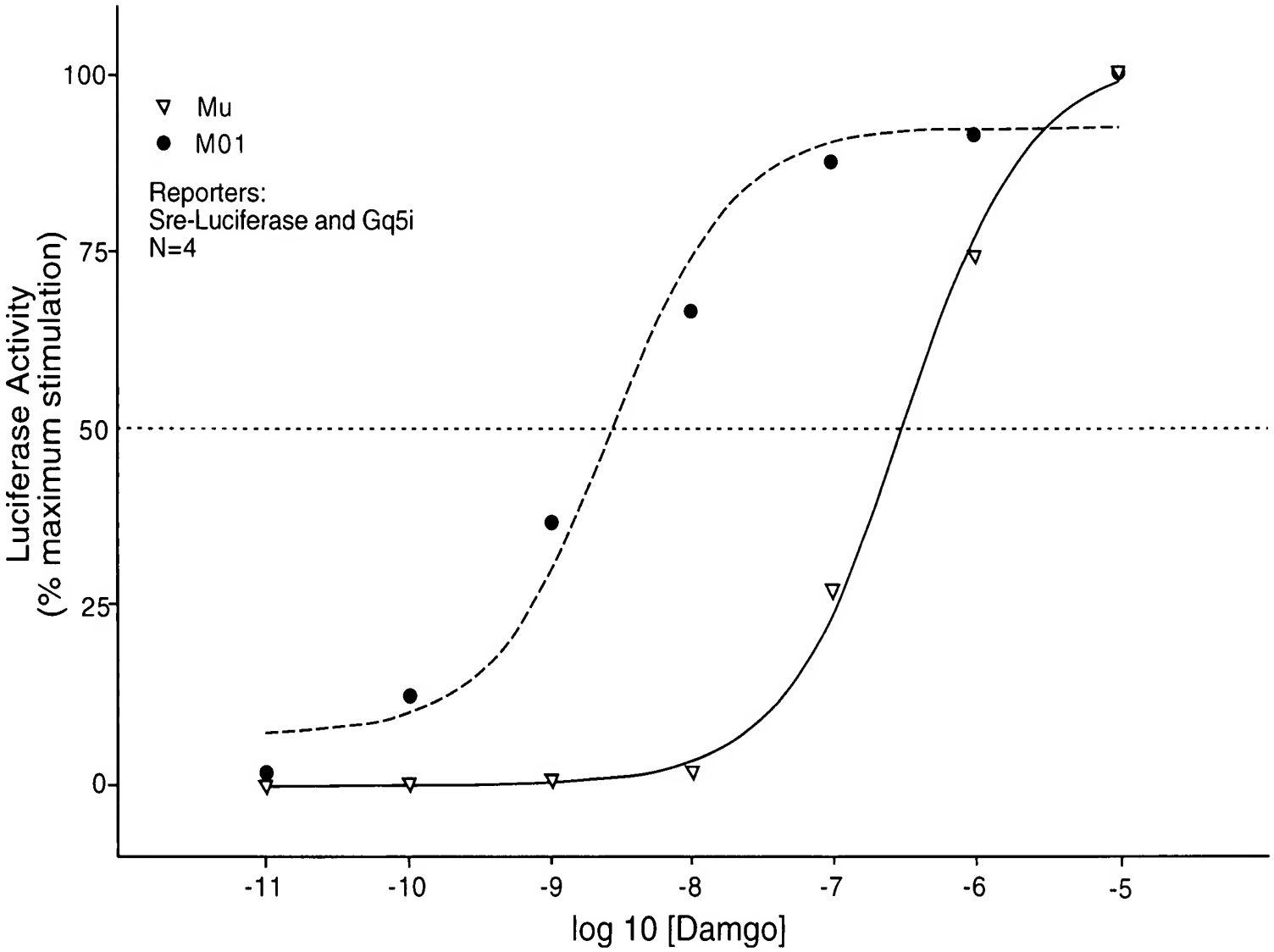


FIG. 16

An Intracellular Point Mutation Results in
Loss of Ligand-Induced Function

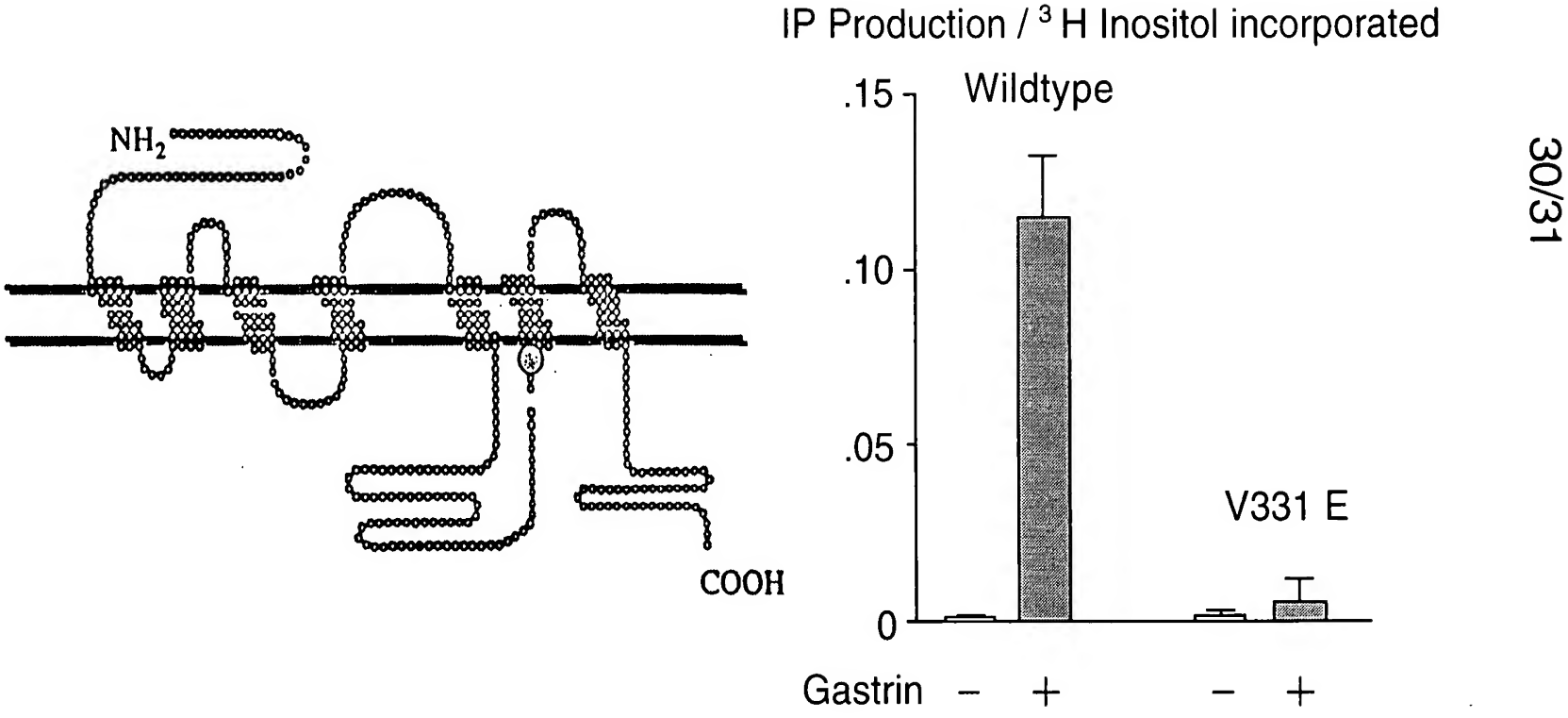
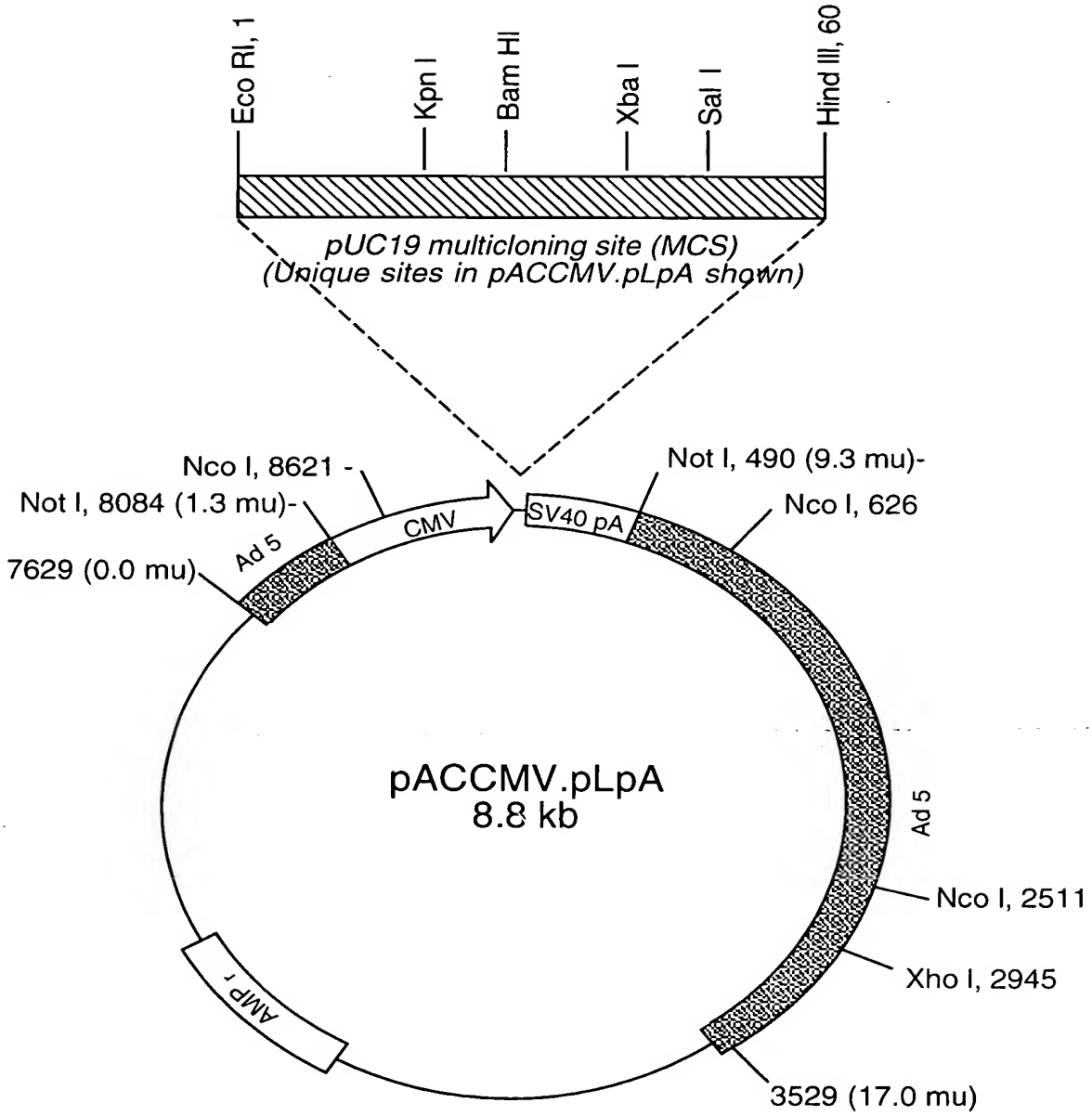


FIG. 17


Eco RI, 1
Kpn I
Bam HI
Xba I
Sal I
Hind III, 60
pUC19 multicloning site (MCS)
(Unique sites in pACCMV.pLpA shown)
Nco I, 8621 -
Not I, 8084 (1.3 mu)-
7629 (0.0 mu)
Ad 5
CMV
SV40 pA
Not I, 490 (9.3 mu)-
Nco I, 626
pACCMV.pLpA
8.8 kb
Ad 5
Nco I, 2511
Xho I, 2945
3529 (17.0 mu)
AMP r